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Application of *Trichoderma* sp. for enhancing growth and defence mechanism of red chilli (*Capsicum annuum* L.) cultivated under aluminum stress

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ABSTRACT

The extensive peatlands across Indonesia offer significant potential for cultivating food and horticultural crops, particularly red chili, a valuable commodity. However, these lands face significant challenges, including low pH levels and the presence of heavy metals, which can hinder plant growth. Heavy metals are harmful environmental pollutants that pose risks to agricultural land and plant health. Plant-microorganism interactions, like those with *Trichoderma* species, can boost plant growth and reduce aluminum (Al) toxicity. This study evaluated the effects of *Trichoderma* sp. on the growth and defense mechanisms of Tanjung variety chili plants under Al stress. The experiment used a Completely Randomized Design in factorial pattern with two factors: *Trichoderma* sp. at four levels (0 g, 10 g, 15 g, 30 g) and Al 0 ppm, 100 ppm, 200 ppm, 300 ppm). Observations included plant height, number of branches, plant dry weight, water content, total chlorophyll content, catalase and ascorbate peroxidase enzymes activity. The results indicated that 300 ppm Al reduced plant height to 14.22 ± cm, while adding 15 g *Trichoderma* sp. and 200 ppm Al increased plant height up to 20.2 ± 1.90 cm (control 15.14 ± 2.75 cm). 300 ppm Al reduced plant dry weight to 3.12 ± 0.12 g, but adding 15 g *Trichoderma* sp. increased plant dry weight to 8.29 ± 1.32 g compared to control (5.14 ± 0.46 g). The treatment of 30 g *Trichoderma* sp. without Al-induced increased total chlorophyll content (3.85 ± 0.9) (control 3.56 ± 0.90). Chili plants showed enhanced defence mechanism responses with higher CAT enzyme activity (27.95 ± 1.31 units/mg) when treated with 15 g of *Trichoderma* sp. and 300 ppm Al stress. Additionally, the application of 10 g of *Trichoderma* sp. under 300 ppm Al stress elevated APX enzyme activity to 0.111 ± 0.007 units/mg, and under 100 ppm Al stress. Thus, the treatment of 15 g and 30 g *Trichoderma* sp. effectively improved chilli plant growth and countered the adverse effects of Al stress.

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1. Introduction

Indonesia is one of the countries that has extensive peatland, covering approximately 13.4 million hectares, with the most widespread distribution in Sumatra and Kalimantan, and a small portion can be found in Papua and Sulawesi (Anda et al., 2021; Pulunggono et al., 2022). There are several heavy metal contents found in peatlands, one of them is the presence of Aluminum (Al), which can increase to toxic levels for plants (Soewandita, 2018; Suwardi, 2019). Al can be absorbed and excessively accumulated by plants if it is extremely available in the soil. High availability of Al can be found in soils with low pH such as peatlands (Suwardi, 2019). It becomes toxic in acidic conditions, where Al^{3+} is a highly toxic form for plants. Al can be absorbed by plants through root tips, and its presence can inhibit root elongation and cell division, resulting in decreased nutrient and mineral absorption. Additionally, Al can also inhibit the photosynthesis process, thus impacting plant growth and yield quality (Panda et al., 2009).

An advantageous method to mitigate the harmful effects of heavy metal Al by the addition of symbiotic microorganisms. The association of microorganisms with plants can be a beneficial alternative in overcoming the toxicity effects of heavy metal Al on plants. This interaction is a strategy that can be employed to reduce toxicity and trigger plant resistance to Al (Junaedi et al., 2021). *Trichoderma* sp. is one of the microorganisms that can symbiotically associate with plants and can be utilized to mitigate heavy metal toxicity while stimulating plant growth (Singh et al., 2019). It has main activities including inducing plants against phytopathogens, acting as biofungicides, and other abilities such as enhancing resistance to environmental stresses and promoting plant growth and development (Mazhabi et al., 2011; Fazeli-Nasab et al., 2022).

In agriculture, *Trichoderma* sp. has been used and is highly beneficial in reducing the negative effects of pathogens and enhancing plant defense against abiotic stresses such as drought, salinity, extreme temperatures, and heavy metal stress (Hidangmanyum and Dwivedi, 2017). *Trichoderma* sp. can stimulate root growth and increase water and nutrient uptake under stress conditions. Alongside these abilities, secondary metabolites and enzymes are also produced by *Trichoderma* sp. to enhance

physiological processes and protect plants from various environmental stresses.

In plant root systems, the application of *Trichoderma* sp. plays an important role in helping to improve soil nutrition, thus enhancing nitrogen use efficiency which affects plant signaling cascade activation (Singh et al., 2019). *Trichoderma* sp. is a microbe belonging to Plant Growth Promoting Rhizobacteria (PGPR) as biological fertilizer that can also increase the activity and diversity of the rhizosphere microbiome and stimulate the secretion of chemical compounds (Liu et al., 2021; Asriatno et al., 2023). Research conducted by Nongmaithem et al. (2017) yielded results showing that paddy plants (*Oryza sativa* L.) treated with *Trichoderma* sp. experienced increased biomass production along with a decrease in the concentration of the heavy metal Cadmium (Cd) in paddy plants. In environments contaminated with heavy metals, *Trichoderma* sp. not only demonstrates high tolerance but also has the ability to accumulate significant amounts of heavy metals. Its capability to produce metal-chelating compounds plays a crucial role in mitigating heavy metal contamination, making *Trichoderma* sp. a promising eco-friendly bioremediation agent for reducing heavy metal concentrations in the environment (Nongmaithem et al., 2017; Yadav et al., 2024; Altaf et al., 2024).

Red chili (*Capsicum annuum* L.) is a widely cultivated horticultural plant with high economic and nutritional value. One potential cultivation area for red chili is peatland, which, despite its challenging conditions, can be utilized for agricultural purposes. However, peatland and other acidic soils often contain high concentrations of heavy metals, including lead (Pb), chromium (Cr), cadmium (Cd), zinc (Zn) (Nurcahaya et al., 2019), and aluminum (Al) (Andriyani and Jadid, 2021), which can negatively impact plant growth and productivity. To mitigate the toxic effects of Al stress, the use of beneficial microbes such as *Trichoderma* sp. has gained attention due to its ability to enhance soil health and plant resilience in low-pH environments (Mishra et al., 2022). This study aims to evaluate the role of *Trichoderma* sp. in supporting the growth and yield of Tanjung variety red chili under aluminum stress. The results are expected to provide valuable insights and recommendations for optimizing red chili cultivation on peatlands and other acidic soils.

2. Materials and methods

2.1. Materials

Chilli seed plants of the Tanjung variety obtained from the Balai Penelitian Tanaman Sayuran (BALITSA) in Lembang. Commercial *Trichoderma* sp. in a powder form while Aluminium in the form AlCl3 solutions were given at various concentrations as mentioned below. The growing media consisted of rice husk charcoal, cocopeat, and rockwool, totaling 3 kg planted in polybags measuring 17.5 cm (diameters) x 35 cm (length). Mutiara NPK fertilizer (16:16:16) The equipment used in this research were, Oven, SPAD chlorophyll meter and Spectrophotometer UV-Vis.

2.2. Planting and treatments

The research was conducted at the SITH ITB greenhouse and laboratory from March to September 2023. *Trichoderma* sp. was applied at various levels, 0 g as a control, 10 g, 15 g, and 30 g, were applied during seeding, with the addition of 5 g in each treatment, and then applied to the growing media simultaneously with planting the seedlings in polybags. Aluminum was given in the form of $AlCl_3$ at various levels: 0 ppm as a control, 100 ppm, 200 ppm, and 300 ppm. The application of *Trichoderma* sp. and Al was combined at varying concentrations above to obtain the treatment combinations shown in Table 1. The adjusment of pH of the growing media was achieved with CH_3COOH to a range of pH 5.0-5.6 (Andriyani and Jadid, 2021). Mutiara NPK fertilizer (16:16:16) was applied 14 days after planting (DAP) at a concentration of 2.5 g per plant, with additional fertilizer application at 56 days after planting (DAP) (Fitria et al., 2021). The number of plants used per treatment are 5 replicates with 16 treatment combinations, resulting in a total of 80 plants.

Table 1. Combination treatment of *Trichoderma* sp. and Al stress to red chili plants

Aluminium	*Trichoderma* sp.			
	T_0	T_1	T_2	T_3
A_0	A_0T_0	A_0T_1	A_0T_2	A_0T_3
A_1	A_1T_0	A_1T_1	A_1T_2	A_1T_3
A_2	A_2T_0	A_2T_1	A_2T_2	A_2T_3
A_3	A_3T_0	A_3T_1	A_3T_2	A_3T_3

2.3. Measured parameters

Observations were conducted on several growth parameters, including plant height, number of branches, plant dry weight, total chlorophyll content, number of flowers, fruit yield, and fruit weight. Plant height observations were made every week (5 WAP), while observations on the number of branches, measurements of plant dry weight and water content were performed at the end of the growing period. Chlorophyll content was measured during the plant growth period, and chilli fruit harvesting was carried out over one month (July-August) with eight harvesting time. Catalase (CAT) and Peroxidase Enzyme (APX) activity analyzed using spectrophotometer UV-VIS.

2.4. Chlorophyll content analysis (addition)

Leaf chlorophyll content was measured during the vegetative phase by assessing the upper or young leaves of the plant. Three leaves were measured per plant, with three measurement points on each leaf using a SPAD Chlorophyll Meter, and the results were then averaged. The obtained chlorophyll units were subsequently converted using a UV-Vis spectrophotometer, following the method of Quinet et al. (2012). A total of 0.1 g of young leaves was homogenized (ground in a chilled mortar) with 10 mL of 80% acetone. The extract was then centrifuged for 10 minutes at 3000 rpm at 4°C to separate the pellet and supernatant. The obtained supernatant was used to measure photosynthetic pigments using a UV-Vis spectrophotometer at wavelengths of 663 nm and 645 nm.

2.5. Catalase (CAT) and ascorbate peroxidase (APX) enzyme analysis (addition)

The analysis of catalase (CAT) and ascorbate peroxidase (APX) enzyme activity was conducted 15 days after treatment by collecting fresh plant leaves (Singh et al., 2021). Sample preparation and enzyme activity analysis followed the method of Maksimović and Živanović (2012) as cited in Taufikurahman and Aziz (2021). Sample extraction was performed using a ratio of fresh leaf weight to buffer volume of 1:5. A total of 1 g of fresh leaves was ground in a mortar with liquid nitrogen, followed by the addition of 5 mL of pre-prepared extraction buffer and homogenization. The homogenized sample was then transferred into a centrifuge tube and centrifuged at 13,000 rpm for 10 minutes at 4°C. The obtained filtrate was used as the sample for measuring CAT and APX enzyme activity.

The measurement of CAT enzyme activity in a 1 mL cuvette consisted of 920 µL buffer, 70 µL sample, and 10 µL H_2O_2. Absorbance changes were recorded using a spectrophotometer at a wavelength of 240 nm. Absorbance readings were taken every 30 seconds for three minutes. Enzyme activity was expressed in units, defined as the amount of enzyme decomposing 1 µM H_2O_2 per minute at pH 7 and 25°C. The calculation formula used was as follows:

$$\text{Volume activity (unit/mL)} = \frac{\Delta A \cdot Vq}{0{,}0436 \cdot Vs}$$

$$\text{Enzyme activity (unit/mL)} = \frac{\text{volume activity}}{\text{fresh weight (FW)}}$$

Ascorbate peroxidase (APX) enzyme activity was measured using a spectrophotometer by monitoring the absorbance change at a wavelength of 290 nm at 28°C. The reaction mixture in the cuvette contained 890 µL buffer, 2 µL ascorbic acid, 30 µL sample, and 20 µL H_2O_2. Absorbance readings were taken every 180 seconds after initiating the reaction by adding H_2O_2. Enzyme activity was expressed in units, defined as the amount of enzyme oxidizing 1 µmol of ascorbic acid per minute. The calculation formula used was:

$$\text{Volume activity (unit/mL)} = \frac{\Delta A \cdot 2 \cdot Vq}{2{,}8 \cdot Vs}$$

$$\text{Enzyme activity (unit/mL)} = \frac{\text{volume activity}}{\text{fresh weight (FW)}}$$

ΔA : Change in absorbance
Vq : Reaction volume in the cuvette (mL)
Vs : Sample volume used (mL)
0,0436 : Millimolar extinction coefficient of H_2O_2 at 240 nm
2,8 : Ascorbate extinction coefficient at 290 nm

2.6. Data analysis

This study was conducted using a Completely Randomized Design in factorial pattern. The observation data were analyzed using Two-way Analysis of Variance (ANOVA), followed by Duncan's New Multiple Range Test (DNMRT) at a 5% significance level using SPSS 29 software.

3. Results and discussion

3.1. Plant height

The combination treatment of aluminum and *Trichoderma* sp. showed a statistically significant effect (p-value = 0.035, $p < 0.05$). The treatment with 200 ppm Al and 15 g *Trichoderma* sp. resulted in the highest plant height, reaching 19.72 cm, compared to the control (15 cm) (Fig. 1). In contrast, the lowest plant height (14.22 cm) was observed in the treatment with 300 ppm Al and 0 g *Trichoderma* sp. These statistically significant results suggest that the combination of 200 ppm Al and 15 g *Trichoderma* sp. can enhance plant growth, whereas the 300 ppm Al treatment may inhibit chili plant growth. Additionally, an interaction was observed between *Trichoderma* sp. treatment and chili plant response under Al stress (Fig. 2). These results can also be observed through the differences in plant height among treatments at 105 days after planting (DAP) (Fig. 3).

Previous research by Maldaner et al. (2020) showed that there was an increase in the height of *Schinus molle* (L.) by *Trichoderma* sp. treatment and Al in a concentration of 200 mg L-1). It can be seen that giving *Trichoderma* sp. In plant growth media under heavy metal Al stress conditions, it still helps the growth of chilli plants under stress conditions. Similar results were carried out by Doni et al. (2014), showed that there was an increase in plant height in rice plants with the addition of *Trichoderma* sp. on the growing medium used. In this case, there are mechanisms such as nutrient use efficiency in plants and tolerance to biotic stress mechanisms (Hidangmanyum and Dwivedi, 2017). Similar results were also obtained in corn plants which experienced an increase in plant height and biomass production (Harman et al., 2004).

Trichoderma sp. are recognized as Plant Growth-Promoting Fungi (PGPF) due to their ability to produce siderophores, phosphate-solubilizing enzymes, and phytohormones, which enhance plant growth (Doni et al., 2014; Hidangmanyum and Dwivedi, 2017). The increase in plant height observed with *Trichoderma* sp. application is associated with a higher number of branches (Fig. 4). This effect is likely attributed to the ability of Trichoderma sp. to enhance nitrogen fixation, thereby improving nitrogen uptake, although this study did not specifically analyze nitrogen content. According to Singh et al. (2019), the interaction between *Trichoderma* sp. and plant roots involves nitrate transporters that facilitate nitrogen absorption into the root system. Colonization of *Trichoderma* sp. on plant roots releases compounds that activate nitrogen receptors on the root surface, triggering a signaling response to nitrate transporters. This process optimizes nitrogen absorption from the roots to all parts of the plant. Additionally, nutrients are supplied in the growing medium during the vegetative period, further supporting plant development (Tyśkiewicz et al., 2022).

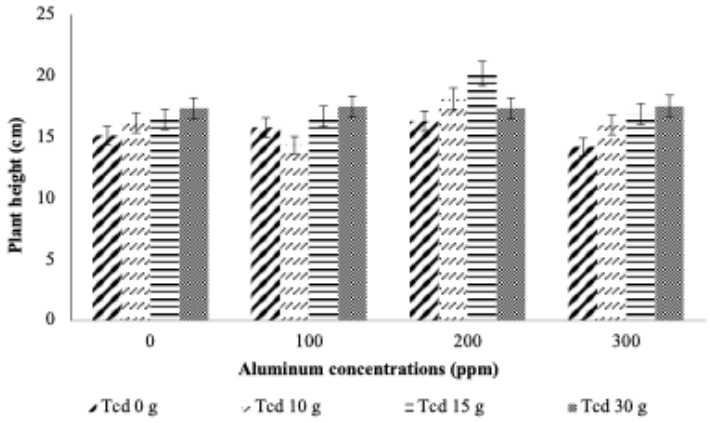

Fig. 1. Red chilli plant height with treatment of *Trichoderma* sp. under Al stress. Numbers followed by the same lowercase letters in the same bar are not significantly different in the DNMRT Test at the 5% level.).

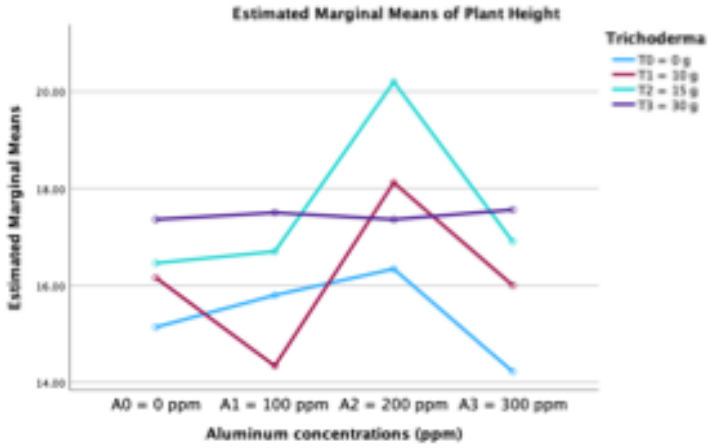

Fig. 2. Interaction effect model of *Trichoderma* sp. treatment and Al stress to red chili plant height.

Fig. 3. Red chilli plant height with treatment of *Trichoderma* sp. under Al stress (A0 = Al 0 ppm, A1 = Al 100 ppm, A2 = 200 ppm, A3 = 300 ppm; T0 = *Trichoderma* 0 g, T1 = 10 g, T2 = 15 g, T3 = 30 g. Numbers followed by the same lowercase letters in the same bar are not significantly different in the DNMRT Test at the 5% level.).

3.2. Number of branches

The treatment of *Trichoderma* sp. did not show statistically significant results for the number of branches parameter in red chilli plants under Al stress ($p \geq 0.05$) (Fig. 3). However, the results also indicate a trend of increased branch number under the application of *Trichoderma* sp. at 30 g and aluminum (Al) stress at a concentration of 300 ppm.

A reduction in the number of branches in chilli plants was observed with the Al treatment when *Trichoderma* sp. was not added. The lowest result, with an average of 13.6 branches, was seen in chilli plants treated with 300 ppm Al without *Trichoderma* sp. Adding *Trichoderma* sp. to the growing medium can enhance vegetative growth, as indicated by an increase in the number of branches with higher doses of *Trichoderma* sp. Conversely, the application of Al led to a decrease in branch growth as the Al concentration increased. This suggests that Al stress can hinder the vegetative growth of chilli plants, although it does not reach a level that would kill the plants.

Trichoderma sp. has shown a significant positive effect on increasing the number of branches, even when plants are grown under heavy metal stress such as Al. This is attributed to the ability of *Trichoderma* sp. to chelate heavy metals and enhance nutrient availability and absorption in plants, thereby improving physiological functions (Yadav et al., 2024). In this study, the application of *Trichoderma* sp. to chili plants under Al-untreated stress conditions effectively increased branch formation. This finding aligns with the study by Prisa (2020), which reported that *T. viride* not only stimulates cell elongation but also promotes new branch formation and leaf development, thereby enhancing the vegetative parameters of *Kalanchoe pinnata*, *K. tubiflora*, and *K. gastonis-bonnieri*. Additionally, *Arabidopsis thaliana* inoculated with *T. atroviride* IMI 206040 in an in vitro culture exhibited an increase in branch formation and lateral root development (Cornejo-Rios et al., 2022).

The application of *Trichoderma* sp. under Al stress conditions can influence the number of plant branches. *Trichoderma* sp. is capable of producing organic acids that lower soil pH, facilitating the solubilization of phosphate (P) and other macro and micronutrients. This enhances the availability of essential nutrients required for plant growth and development, including branch formation (Shetty et al., 2021). Consequently, the application of *Trichoderma* sp. can increase branch formation in plants subjected to Al stress. *Trichoderma* sp. produces organic acids such as gluconic acid, fumaric acid, and citric acid, which reduce soil pH and promote the solubilization of phosphate and essential macro- and micronutrients, including iron, manganese, and magnesium, which are crucial for plant metabolism (Kacprzak et al., 2014).

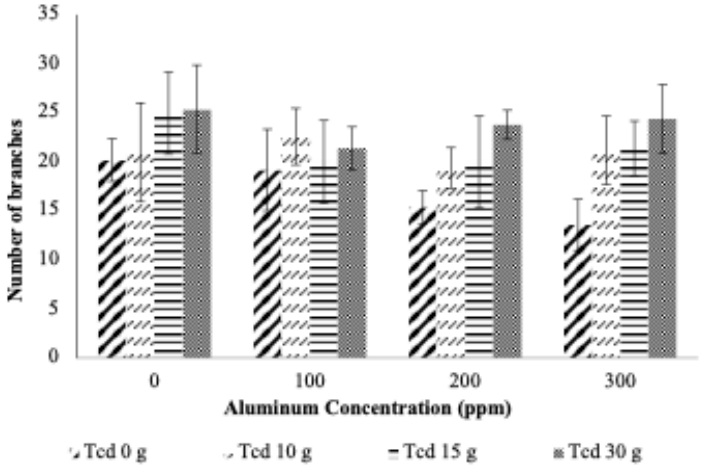

Fig. 4. Number of branches of red chilli plants with treatment of *Trichoderma* sp. under Al stress. (Tcd = *Trichoderma* sp.).

3.3. Plant dry weight

Treatment of Al with various concentrations tends to reduce the dry weight of chilli plants (Fig. 5). There were statistically significant results from the combination of 300 ppm Al with 15 g *Trichoderma* (8.29 g) on the plant dry weight parameters of chilli plants compared to control (5.14 g). The addition of *Trichoderma* tends to increase the dry weight of plants which is related to photosynthate accumulation. Based on these results, it can be assumed that the addition of 15 g of *Trichoderma* can increase the dry weight of the plant even under Al stress.

The increase in Al concentration (200 and 300 ppm) leads to a reduction in the dry weight of red chili plants. This decline is primarily due to Al-induced stress, which disrupts water and nutrient uptake, resulting in nutrient deficiencies, reduced photosynthesis, and overall lower biomass accumulation. However, the combination of Al and *Trichoderma* sp. has been found to improve plant dry weight by enhancing both shoot and root growth. In contrast, Al stress in plants not treated with *Trichoderma* sp. further exacerbates the decrease in dry weight as Al concentration increases. Similar findings were reported by Meneses et al. (2022), who observed a decrease in plant dry weight with increasing Al concentration and *Trichoderma* sp. application. Additionally, comparable results were found in plants subjected to various abiotic stresses, such as salinity, cold temperatures, drought, and excess water (Cornejo-Ríos et al., 2021; Elkelish et al., 2020). Meanwhile, the application of *Trichoderma* sp. can positively influence plant biomass by promoting the availability of indole-3-acetic acid (IAA), an auxin hormone that stimulates root growth and elongation,

leading to more efficient nutrient absorption (Meneses et al., 2022). The interaction between plant root systems and microorganisms in the rhizosphere plays a crucial role in sustaining plant growth and productivity (Etesami and Maheshwari, 2018).

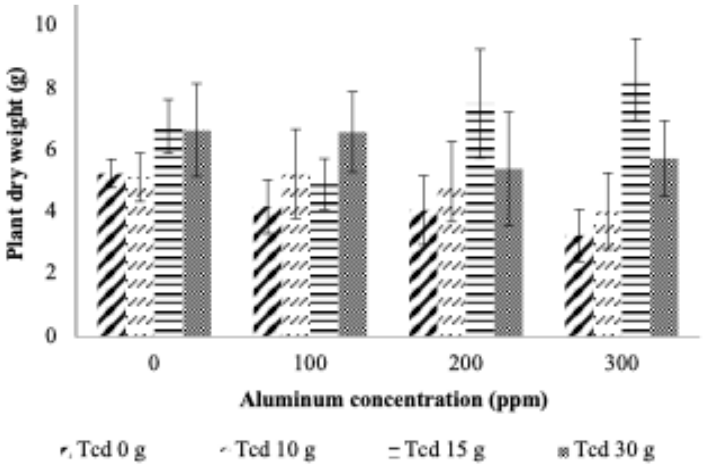

Fig. 5. Plant dry weight (g) of red chilli plants with treatment of *Trichoderma* sp. under Al stress. (Tcd = *Trichoderma* sp.).

Trichoderma sp. are known to produce siderophores, which act as heavy metal chelating agents to reduce heavy metal toxicity. In addition, they can increase plant height, thereby enhancing plant biomass, as observed in research by Yadav et al. (2024) on tomato seedlings exposed to high levels of heavy metals. The accumulation of biomass in plants due to *Trichoderma* sp. application supports water and nutrient absorption and mitigates the effects of Al stress (Eslahi et al., 2020).

3.4. Water content

The application of *Trichoderma* sp. did not significantly affect the water content of chili plants grown under Al stress compared to the control ($p > 0.05$) (Fig. 6). However, a significant reduction in water content was observed in plants subjected to 300 ppm Al stress without application of *Trichoderma* sp. with a decrease to 17.88 g, which was notably lower than the control (38.43 g). In contrast, the combination of Al concentrations and *Trichoderma* sp. did not significantly affect water content of red chilli plants. The decrease in plant water content under Al stress at 200 and 300 ppm can be attributed to several physiological disruptions caused by aluminum toxicity. Aluminum stress negatively affects root development by inhibiting root elongation and damaging root cell membranes, which in turn reduces the plant's ability to absorb water efficiently.

This result is in accordance with research by Guo et al. (2018), who stated that giving 1 mM Al could reduce the water content of citrus plants (*Citrus grandis*) by 60% compared to the control (90%). Kochian et al. (2015) stated that plant exposure to Al toxicity could trigger water stress, especially physiological drought, which limits the plant's capacity to obtain water and nutrients. This result can occur because the concentration of 300 ppm is the highest concentration used, which causes a decrease in the capacity of chilli plants to absorb and retain the water content in them.

According to Ofoe et al. (2023), this condition can be caused by cell damage and disruption of plasma membrane function, which will affect the flow of ions to essential parts of the plant for physiological processes (Gupta et al., 2013). Active transport of water and nutrients is triggered by a hydrogen ion gradient, which is also influenced by proton pumps in the plasma membrane (Zhang et al., 2018). Al compounds will bind to the phospholipid layer in the plasma membrane, which can reduce the balance of membrane potential and inhibit the activity of H+-ATPase protons from leaving the plasma membrane, thereby causing water and nutrient transport to be disrupted (Zhang et al., 2017).

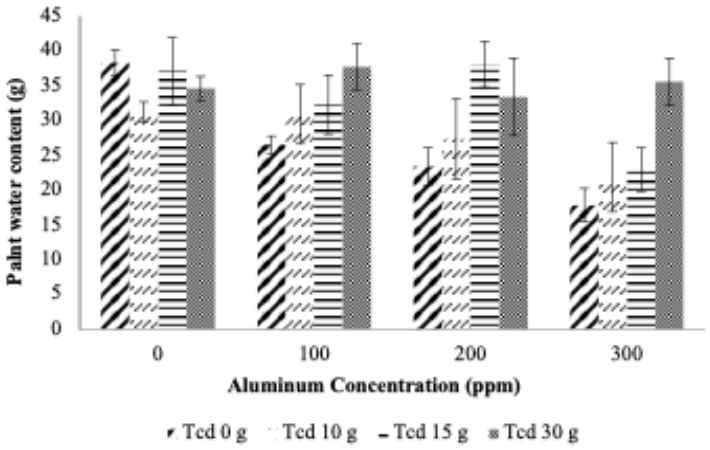

Fig. 6. Water content of red chilli plants with treatment of *Trichoderma* sp. under Al stress. Different letters indicate significantly different results based on the Duncan Test at the 5% level ($P \leq 0.05$). (Tcd = *Trichoderma* sp.).

Zishiri et al. (2022) stated that the decreased water content in plants due to the direct effects of Al is related to the inhibition of root elongation, which makes it difficult for the roots to absorb water and nutrients optimally. This is evidenced by the decrease in plant root length when 300 ppm Al is applied (Fig. 6). According to Ofoe et al. (2023), impaired root growth and decreased root volume due to high Al concentrations in the soil can disrupt water and nutrient absorption. When absorbed by plants, Al inhibits root cell elongation, leading to a reduction in root biomass. Additionally, symptoms of root atrophy or root rot can further reduce water content in plants (Chauhan et al., 2021; Meneses et al., 2022).

3.5. Total chlorophyll content

The combination treatment of Aluminum and *Trichoderma* sp. did not show statistically significant results ($p > 0.05$) on chlorophyll contents in chilli plant leaves (Fig. 7). However, the results of chlorophyll levels showed a tendency to increase with increasing doses of *Trichoderma* sp. in plants grown without Al stress. It was proven that giving 30 g of Trichoderma could increase chlorophyll levels up to 3.85 mg/g and was significantly different from the control (3.56 mg/g) (Fig. 8).

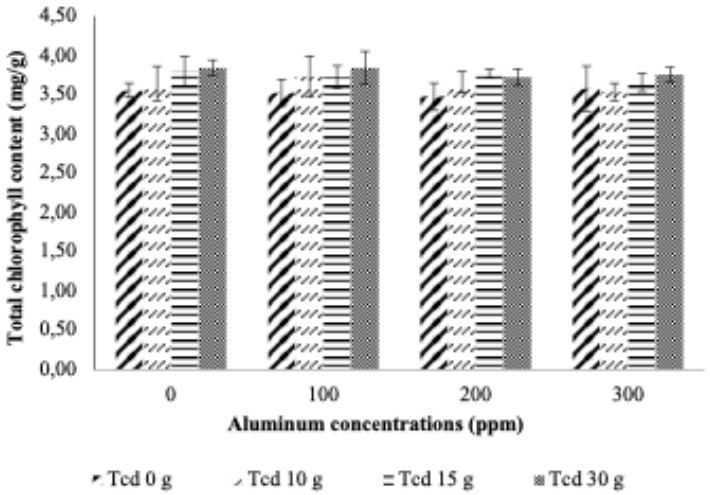

Fig. 7. Chlorophyll content of red chilli plants with treatment of *Trichoderma* sp. under Al stress. (Tcd = *Trichoderma* sp.).

Study by Meneses et al. (2022), showed that there was an increase in the total chlorophyll concentration in maize (*Zea mays* L.) with the combination of *T. asperelloides* and 50 µM Al. Additionally, the application of *T. asperelloides* alone to maize

grown without Al stress resulted in a higher total chlorophyll concentration (1.5 mg/g^{-1} FW compared to maize under Al stress. In the study by Meneses et al. (2022), there was an increase in the total chlorophyll concentration in maize with the combination of *T. asperelloides* and Al at concentrations of 50 µM and 100 µM. Furthermore, the application of *T. asperelloides* alone maize grown without Al stress resulted in a higher total chlorophyll concentration compared to corn plants under Al stress.

Pehlivan et al. (2017) stated that photosynthetic pigments were correlated with increased photosynthesis rates in corn plants inoculated with *Trichoderma lixii*. Although photosynthesis rates were not measured in this study, they are related to chlorophyll levels in the leaves. Inoculation with *Trichoderma* sp. isolates can increase chlorophyll levels and other photosynthetic pigments, thereby enhancing the rate of photosynthesis in plants under Al stress (Harman et al., 2019; Meneses et al., 2022). Based on these results, 200 ppm Al stress affects the reduction of total chlorophyll levels. According to Chen et al. (2020), the most significant toxic effect of Al on leaf cell ultrastructure is chloroplast damage. Al toxicity causes a loss of chloroplast integrity, as indicated by the loss of chloroplast membranes and distortion of grana lamellae.

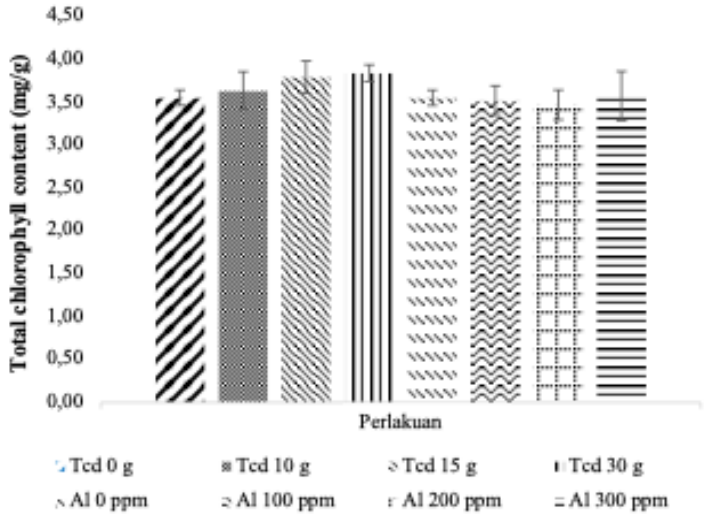

Fig. 8. Chlorophyll content of red chilli plants with treatment of *Trichoderma* sp. and under Al stress. Different letters indicate significantly different results based on the Duncan Test at the 5% level ($P \leq 0.05$). (Tcd = *Trichoderma* sp.).

3.6. Catalase (CAT) and ascorbate peroxidase (APX) enzymes activity

Catalase (CAT) enzyme activity is an enzyme that is closely related to plant defense mechanisms against biotic stress. The results obtained show that the activity of the CAT enzyme increases with increasing Al concentration (Fig. 9). The combination of Al and *Trichoderma* sp. showed statistically significant results on CAT activity. The treatment of 15 g *Trichoderma* sp. and 300 ppm Al stress showed the highest CAT activity (27,95 unit/mg) compared to the untreated plant (8,89 unit/mg). Based on the findings of this study, it is suspected that *Trichoderma* sp. not only aids plants in mitigating the negative effects of aluminum (Al) stress but also enhances the plant's antioxidant response, thereby increasing CAT enzyme activity. This finding is consistent with the study by He et al. (2021), which reported that the application of 5 mL *Trichoderma* sp. on maize seedling leaves under cadmium (Cd) stress at 200 mg/kg increased CAT enzyme activity to 4,500 units/g/min, compared to 1,500 units/g/min in Cd-stressed plants without *Trichoderma* sp. treatment. Additionally, the study by Altaf et al. (2022) demonstrated an increase in CAT enzyme activity with rising nickel (Ni) concentrations (20-100 mg/L) in chili plants, with the highest CAT activity observed at 100 mg/L Ni treatment, reaching 0.43 units/g compared to the control (0.10 units/g).

Apart from the CAT enzyme, there are other enzymes that can be used to measure enzyme activity in plants under biotic stress, namely Ascorbate Peroxidase (APX). In terms of APX enzyme activity, the application of *Trichoderma* sp. significantly enhanced APX enzyme activity (Fig. 10). This effect was observed in chili plants treated with 10 g of *Trichoderma* sp. and subjected to 300 ppm Al stress, which exhibited a APX enzyme activity of 0.111 units/mg compared to the control without *Trichoderma* sp. and Al stress (0.049 units/mg). However, this result was not significantly different from the treatment with 300 ppm Al without *Trichoderma* sp. application (negative control), which recorded an enzyme activity of 0.101 units/mg.

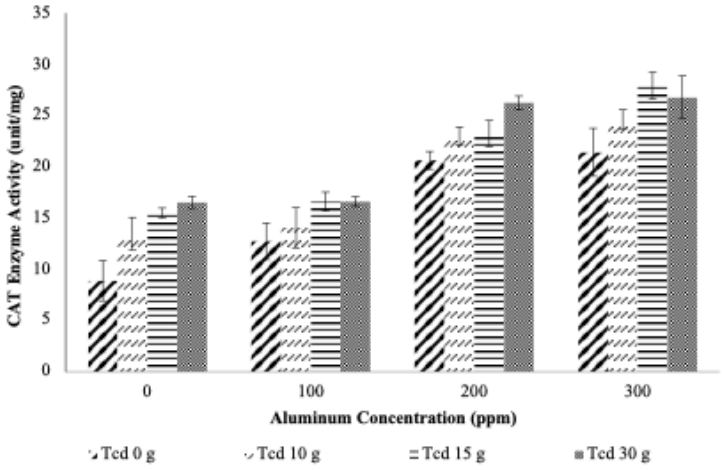

Fig. 9. Catalase (CAT) enzyme activity of red chilli plants with treatment of *Trichoderma* sp. and under Al stress. Results indicate not significantly different based on the Duncan Test at the 5% level ($P \leq 0.05$). (Tcd = *Trichoderma* sp.).

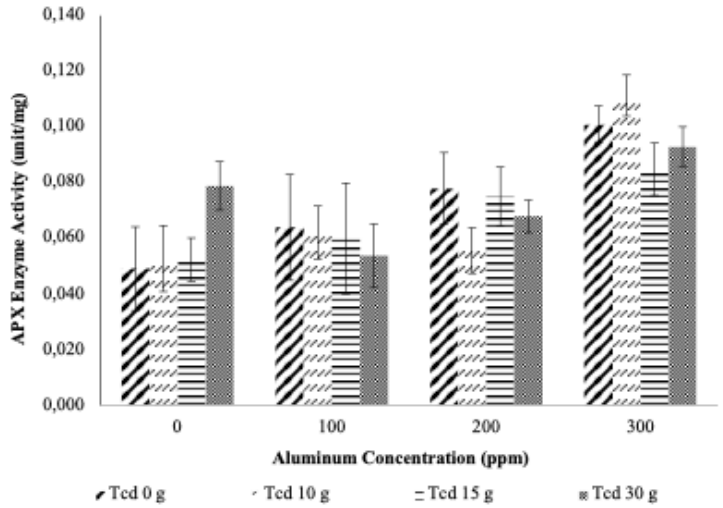

Fig. 10. Ascorbate Peroxidase (APX) enzyme activity of red chilli plants with treatment of *Trichoderma* sp. and under Al stress. Results indicate not significantly different based on the Duncan Test at the 5% level ($P \leq 0.05$). (Tcd = *Trichoderma* sp.).

The enzymatic activity in the treatment with 100 ppm Al combined with *Trichoderma* sp. exhibited a decline, which is likely due to the plant's need to adapt to Al stress and the initial colonization of *Trichoderma* during the early stages of treatment, leading to a temporary reduction in enzyme activity. Additionally, the application of *Trichoderma* sp. itself may contribute to the decrease in enzymatic activity. According to Arif et al. (2016), oxidative damage to cellular structures is a direct and detrimental consequence of exposure to higher concentrations of heavy metals. Moreover, excessive heavy metal stress can also impair the function

of *Trichoderma* sp. in plant growth, potentially inhibiting essential enzymatic activities in plant metabolism (Shi et al., 2022).

At high concentrations of heavy metals, an accumulation of antioxidant enzymes generally occurs (Altaf et al., 2024). One of the key tolerance mechanisms in plants to mitigate reactive oxygen species (ROS) involves enhancing the activity of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX), which have been shown to increase under heavy metal stress conditions (Remírez-Valdespino and Orrantia-Borunda, 2021). The reduction of H_2O_2 by APX is mediated through the use of ascorbic acid as an electron donor, representing the first step in the ascorbate-glutathione cycle (Sharma et al., 2012). *Trichoderma* is capable of producing chelating compounds that bind Al in the rhizosphere, reducing its bioavailability and alleviating oxidative stress. This stress reduction may also contribute to the enhanced activity of the antioxidant enzymes CAT and APX (Yadav et al., 2024).

Table 2. Conclusion of the *Trichoderma* sp. treatment to red chilli plants under Al stress

Parameters	Aluminum	*Trichoderma*	Aluminium and *Trichoderma*
Plant height	***	***	*
Number of branches	***	***	ns
Plant dry weight	ns	***	**
Water content	*	***	***
Chlorophyll content	ns	***	ns
CAT activity	***	***	ns
APX activity	***	ns	***

According according to DNMRT test, the results showed that are followed by notations: ($p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***).

4. Conclusion

Chilli plants showed a notable tolerance to the heavy metal aluminum (Al) based on various growth parametes and defence mechanism. Even at a concentration of 300 ppm, chilli plants can still survive, as indicated by parameters such as plant height, plant dry weight, number of branches water content and and enzymes activity which increase with higher concentrations of Al and *Trichoderma* sp. The presence of Al can trigger plant defence mechanisms, although it may reduce growth parameters such as plant dry weight and water content. Application of *Trichoderma* sp. can mitigate the negative effects of increased Aluminium concentrations on chilli plant growth. Growth parameters like plant height, number of branches, and nu benefit from *Trichoderma* application. This treatment helps chilli plants withstand metal stress from varying aluminum concentrations and supports their productivity. Consequently, chilli plants are suitable for cultivation in acidic soils such as marshes or peatlands. The ability of chilli plants to survive under 300 ppm aluminum stress underscores their resilience.

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Conflict of interest

The authors declare no conflict of interest in this research.

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